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Stromal-Cell Derived Factor Is Expressed by Dendritic Cells and Endothelium in Human Skin

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Stromal-cell derived factor or SDF-1 is a CXC chemokine constitutively expressed by stromal bone marrow cell cultures that binds to the G-protein-coupled receptor CXCR4. SDF-1/CXCR4 represents a unique, nonpromiscuous ligand/receptor pair that plays an essential role in prenatal myelo- and lymphopoiesis as well as in cardiovascular and neural development. SDF-1 prevents entry of CXCR4-dependent (X4) HIV viruses in T lymphocytes, by binding and internalizing CXCR4. The expression pattern of SDF-1 protein in normal tissues is not known. Here we describe an analysis of SDF-1 mRNA and protein in normal and inflamed skin by *in situ* hybridization and immunohistochemistry, using a novel anti-SDF-1 monoclonal antibody. We also describe the expression pattern of CXCR4 receptor by immunohistochemistry. Our results show that SDF-1 protein and mRNA are normally expressed by endothelial cells, pericytes, and either resident or explanted CD1a+ dendritic cells. Epithelial cells of sweat glands but not keratinocytes also express SDF-1. In various inflammatory skin diseases, a large number of mononuclear cells and fibroblasts in close contact with CXCR4-positive lymphocytic infiltrates also express SDF-1. CXCR4 was also detected in many different normal cell types, including endothelial and epithelial cells, which points to a role for SDF-1/CXCR4 cell signaling in vascular and epithelial homeostasis. The demonstration of SDF-1 expression in dendritic and endothelial cells provides new insights into the mechanisms of normal and pathological lymphocyte circulation and makes it possible to envisage a role for locally secreted SDF-1 in the selective incapacity of mucosal dendritic cells to support and propagate infection by X4 HIV isolates. *(Am J Pathol 1999, 155:1577–1586)*

Chemokines are a large family of small peptides with chemoattractant properties.[1] Based on the arrangement of the two first cysteines, they are classified into two main subfamilies, CC and CXC chemokines. Stromal-cell-derived factor 1 (SDF-1) is a CXC chemokine constitutively expressed by bone marrow stromal cells that binds to the G-protein-coupled receptor CXCR4.[2] Two products, α and β, are generated from the SDF-1 gene by alternative splicing. β and α forms differ by the presence of four additional amino acids at the carboxy terminal end of the former.[3,4]

SDF-1/CXCR4 interactions are unique and nonpromiscuous. In mice, SDF-1 or CXCR4 gene knock-outs generate a similar phenotype, characterized by deficient B lympho- and myelopoiesis, and abnormal neuronal and cardiovascular development.[5–8] Embryo lethality associated with either CXCR4 or SDF-1 gene knock-outs emphasizes the critical and unique role played by their products during development. Prenatal death precludes the use of these animal models to investigate the postnatal physiological functions of these proteins. However, the constitutive expression of SDF-1 on one hand and, on the other hand, the fact that SDF-1/CXCR4 represents a nonredundant cell-signaling system, suggest that this chemokine plays a critical role in lymphocytic circulation and immune surveillance in the postnatal life. *In vitro*, SDF-1 shows potent chemoattractant properties for cells expressing the CXCR4 receptor, such as monocytes, lymphocytes, and CD34+ hematopoietic stem cells.[9–11] Moreover, SDF-1/CXCR4 interactions are involved in homing and circulation of leukocytes during B-cell lymphopoiesis and myelopoiesis.[5–7] The role of SDF-1 and CXCR4 in the homing of CD4 T lymphocytes or CD34+ stem cells to the bone marrow of mice has been demonstrated.[12]

Besides its physiological functions, SDF-1 has the unique capacity, not shared with any other known chemokine, to inhibit the entry of CXCR4-dependent (X4) viruses in T lymphocytes by binding and internalizing CXCR4.[13–16] Although both X4 and CCR5-dependent (R5) viruses are detected in AIDS patients, only R5 HIV isolates are transmitted and propagate during asymp-

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tomatic phases of HIV infection, in contrast to X4 isolates, which become predominant in AIDS patients.[17] A role for SDF-1 in delaying the evolution and mortality related to HIV, presumably associated with interference in the propagation of X4 HIV viruses during acute and chronic/asymptomatic phases of infection, has been proposed.[18] SDF-1 transcripts have been detected in many tissues.[3] However, human blood leukocytes do not express SDF-1,[3] and the cell types involved in SDF-1 production and interference with transmission and propagation of X4 HIV isolates remain to be identified.

In an effort to identify SDF-1-producing cells, we have investigated the expression of SDF-1 products in human skin. Human skin offers an accessible and abundant source of tissue to investigate the phenotypic characteristics of cells involved in immunological and inflammatory responses. Importantly, skin contains Langerhans cells, which are critical in antigen presentation and specific priming of naive T lymphocytes. Immature Langerhans cells show a selective incapacity to support infection by X4 HIV isolates or to fuse with cells expressing X4 HIV envelopes that correlates with a relatively low abundance of CXCR4 on their cell surface.[19,20] Thus the study of SDF-1 and CXCR4 expression in skin could shed light on the role played by this ligand/receptor in immunological homeostasis and dissemination of HIV.

Using a novel and specific anti-SDF-1 monoclonal antibody (mAb), we have analyzed SDF-1 expression in normal and inflamed skin. Because many chemokines can be internalized and presented on their surface by different cell types, particularly by endothelial cells (ECs),[21] we have also studied the expression pattern of SDF-1 at the mRNA level by *in situ* hybridization (ISH) to evaluate whether cell types containing SDF-1 protein are also responsible for *SDF-1* gene expression.

Herein we demonstrate that SDF-1 and its receptor CXCR4 are expressed by ECs, pericytes of adult small capillary blood vessels, and some epithelial cells. Importantly, we show that normal Langerhans cells express SDF-1 and that CXCR4 mononuclear cells colocalize with SDF-1-expressing cells in different skin inflammatory diseases. Overall our findings are compatible with a potential role for SDF-1 in the haptotactical attraction of circulating T lymphocytes by ECs and their interaction with antigen-presenting Langerhans cells.

Materials and Methods

SDF-1 Antibody and cDNA

The anti-SDF-1 K15C mAb (IgG2a) recognizes an epitope encompassed in the amino-terminal end of SDF-1; it was developed by immunizing BALB/c mice with a synthetic polypeptide carrying SDF-1 residues 1–15, where cysteines in the motif C9P10C11 were replaced by serines. The SDF-1β cDNA was isolated from primary human fibroblasts by reverse transcription of total RNA and subsequent polymerase chain reaction (PCR) amplification. The cDNA of SDF-1α was obtained by PCR amplification, using the SDF-1β cDNA as a template.[3,4] Nucleotide sequences of both cDNAs were verified by dideoxy sequencing. For expression in mammalian cells, SDF-1 α and β cDNAs were subcloned in a pcDNA3 plasmid (Invitrogen, Carsbad, CA) that allows transcription from a human cytomegalovirus promoter.

Immunodetection of SDF-1 in Cells and Human Skin

Normal skin was obtained from five healthy individuals undergoing minor surgical interventions. Skin biopsies from five patients with cutaneous lupus, five with dermatomyositis, and five with systemic sclerosis (scleroderma) were also studied. All samples were routinely fixed with formalin and embedded in paraffin. Antigen retrieval was performed by microwave heating (3 $\times$ 5 minutes at 750 W in 1 mmol/L EDTA, pH 8) before immunostaining. Endogenous peroxidase was quenched in 3% H_20_2 in methanol for 20 minutes. Staining was performed following a standard indirect avidin-biotin horseradish peroxidase method (ABC standard; Vector Laboratories, Burlingame, CA). Color was developed with diaminobenzidine (Vector Laboratories). The following antibodies were used: anti-SDF-1 clone K15C at 20 μg/ml, anti-CXCR4 clones 12G5 and 6H8[22] at 15 μg/ml and 2 μg/ml, respectively, and undiluted anti-CD1a MAb 010 (Immunotech S.A., Marseille, France). Controls with normal serum instead of primary antibody were always included. For K15C mAb controls preincubating the antibody with SDF-1 protein were also included. Sections were counterstained with hematoxylin.

To evaluate the specificity of K15C mAb, the immortalized goat fibroblast TIGEF cell line[23] was cotransfected by the phosphate calcium method with 2 μg of EGFP-N1 (Clontech, Palo Alto, CA) and 20 μg of either pcDNA3 SDF-1α or β expression vectors. The pcDNA3 insertless vector was used as the control.

TIGEF cells were spread on glass coverslips, and immunofluorescence was performed 40 hours after transfection. To prevent secretion and to enhance intracellular accumulation of SDF-1 isoforms, cells were treated for the last 4 hours in culture with Brefeldine A (10 μg/ml). Cells were washed and fixed in 3.7% paraformaldehyde in phosphate-buffered saline (PBS), permeabilized as previously described,[15] incubated with the K15C mAb (15 μg/ml) for 1 hour at room temperature, and finally incubated with Cy3-goat anti-mouse IgG (Amersham Life Science, Little Chalfont, UK). Confocal microscopy was performed on a Leica TCS4D instrument. Series of 0.3–0.7-μm intervals were recorded and photographed.

The presence of functional SDF-1 in the culture media of transfected cells was confirmed by its ability to downregulate surface CXCR4 in a Jurkat lymphoblastoid T cell line. CXCR4 endocytosis was evaluated by fluorescent activated cell sorting (FACS) analysis as previously described.[15]

Expression of SDF-1 by transfected cells was also confirmed by Western blot. Cells transfected as indicated above were treated or not for the last 4 hours in culture with Brefeldine A. Cells were collected 48 hours after

transfection, and cytoplasmic proteins (60 μg) were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, blotted onto nitrocellulose filters, and probed with the K15C mAb. The ability of K15C to recognize either recombinant or synthetic SDF-1 isoforms was compared. A horseradish peroxidase-labeled rabbit anti-mouse antibody and the ECL system (Amersham Life Science) were used to reveal SDF-1 isoforms by the specific antibody.

In Situ *Hybridization*

Skin sections were dewaxed and rehydrated in PBS containing 0.1% active diethylpyro carbonate (DEPC). Sections were microwaved for 3 × 5 minutes at 750 W in 1 mmol/L EDTA (pH 8) and postfixed in cold 4% paraformaldehyde in PBS for 10 minutes. After treatment with 0.1% Triton X-100, sections were prehybridized in 5× standard saline citrate (SSC), 50% formamide, and 1× Denhardt's solution and hybridized in the same solution containing 0.8 μg/ml of digoxigenin-UTP-labeled riboprobe for 12 hours at 50°C. Sections were washed in SSC to a final concentration of 0.1 × SSC at 55°C. Hybridized probe was detected by incubation with 1:500 alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim, Mannheim, Germany) 4-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate for 1 hour and color developed with either NBT/BCIP or fast red (Vector Laboratories).

Digoxigenin-UTP-labeled sense and antisense SDF-1β riboprobes were synthesized by *in vitro* transcription of the SDF-1β cDNA cloned in pcDNA3 plasmid. Linearized plasmids were transcribed *in vitro*, using SP6 and T7 polymerases according to the manufacturer's protocol (Boehringer Mannheim). The full-length antisense SDF-1β riboprobe used (300 nucleotides) recognizes SDF-1α and SDF1-β mRNA.

SDF-1 RT-PCR Analysis of Explanted Dendritic Cells

Human skin was obtained from patients undergoing breast plastic surgery. Samples were cleared of fat with fine forceps. Skin explants (5 × 5 mm) were floated dermal side down in 10-cm Petri dishes containing tissue culture media (RPMI 1640 supplemented with 10% fetal calf serum, 2 mmol/L glutamine, 1% penicillin/streptomycin, 10 mmol/L HEPES buffer, 1% nonessential amino acids, and 1 mmol/L sodium pyruvate; all from Life Technologies Laboratories, Charing Falls, OH) for 3 days. The nonadherent migratory cells were gently resuspended, filtered to remove debris, and counted with trypan blue before use. Migratory dendritic cells (DCs) were characterized by their dendritic morphology and CD1a FACS analysis. More than 98% of nonadherent cells were CD1a positive.[24]

For detection of SDF-1 mRNA by reverse transcriptase PCR (RT-PCR), total RNA from human CD1a+ cells, foreskin fibroblast, and peripheral blood mononuclear cells (PBMCs) was extracted and reverse transcribed by standard methods. For each sample an identical amount of RNA was incubated with (RT+) or without (RT−) reverse transcriptase. Amplification of SDF-1 cDNAs was performed by PCR, using the following primers: sense 5′ATGAACGCCAAGGTCGTGGT 3′, antisense 5′CTTGTTTAAAGCTTTCTCCAG GTAC 3′. Aliquots of each sample were amplified with β-actin primers[14] as the cDNA loading control.

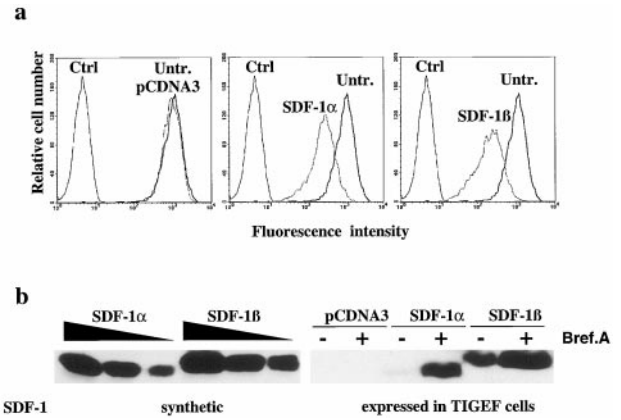

Figure 1. Expression and detection of SDF-1 gene products in mammalian cells by the K15C MAb. **a:** TIGEF cells were transfected with pCDNA3, SDF-1α, or SDF-1β. Forty-eight hours after transfection cell supernatants (pcDNA, SDF-1α, or SDF-1β) were collected, and their ability to promote CXCR4 down-regulation from the surface of lymphoblastoid Jurkat T cells was assessed. Supernatants from untransfected TIGEF cell cultures (Untr.) were used as controls. The expression of CXCR4 was analyzed by cytofluorometry, using the CXCR4-antibody 12G5 or an isotype-matched mouse immunoglobulin (Ctrl). **b:** TIGEF cells were transfected as indicated above and treated or not for the last 4 hours in culture with Brefeldine A (Bref. A). Cells were collected 48 hours after transfection, and cytoplasmic proteins (60 μg) were analyzed by Western blot with the K15C antibody. The ability of the K15C mAb to recognize TIGEF-expressed or synthetic (100, 50, or 25 ng) SDF-1 isoforms was compared.

Results

Specificity of Anti-SDF-1 K15C Antibody

SDF-1 α and β proteins diverge by the presence of four additional amino acids at the carboxy-terminal end in the β isoform. The K15C mAb was generated against a modified amino-terminal-end antigen shared by SDF-1 α and β. The specific recognition of both SDF-1 isoforms by this antibody was assessed by transfecting expression vectors carrying their respective cDNAs in TIGEF cells, which lack detectable SDF-1 mRNA, as assessed by RT-PCR (data not shown). Transfection of TIGEF cells with either SDF-1 α or β cDNA leads to the secretion of active SDF-1 that is capable, as expected,[15,16] of promoting internalization of CXCR4 in a T-cell line (Figure 1a). After cell disruption, cytoplasmic proteins from SDF-1-transfected TIGEF cells were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, blotted, and probed with the K15C mAb. While no signal was detected in lysates from the control, untransfected cells, the antibody recognized molecular species migrating with the expected molecular weight of either SDF-1 α and β (~8 kd). The use of Brefeldine A inhibited secretion and promoted intracellular accumulation of both forms of

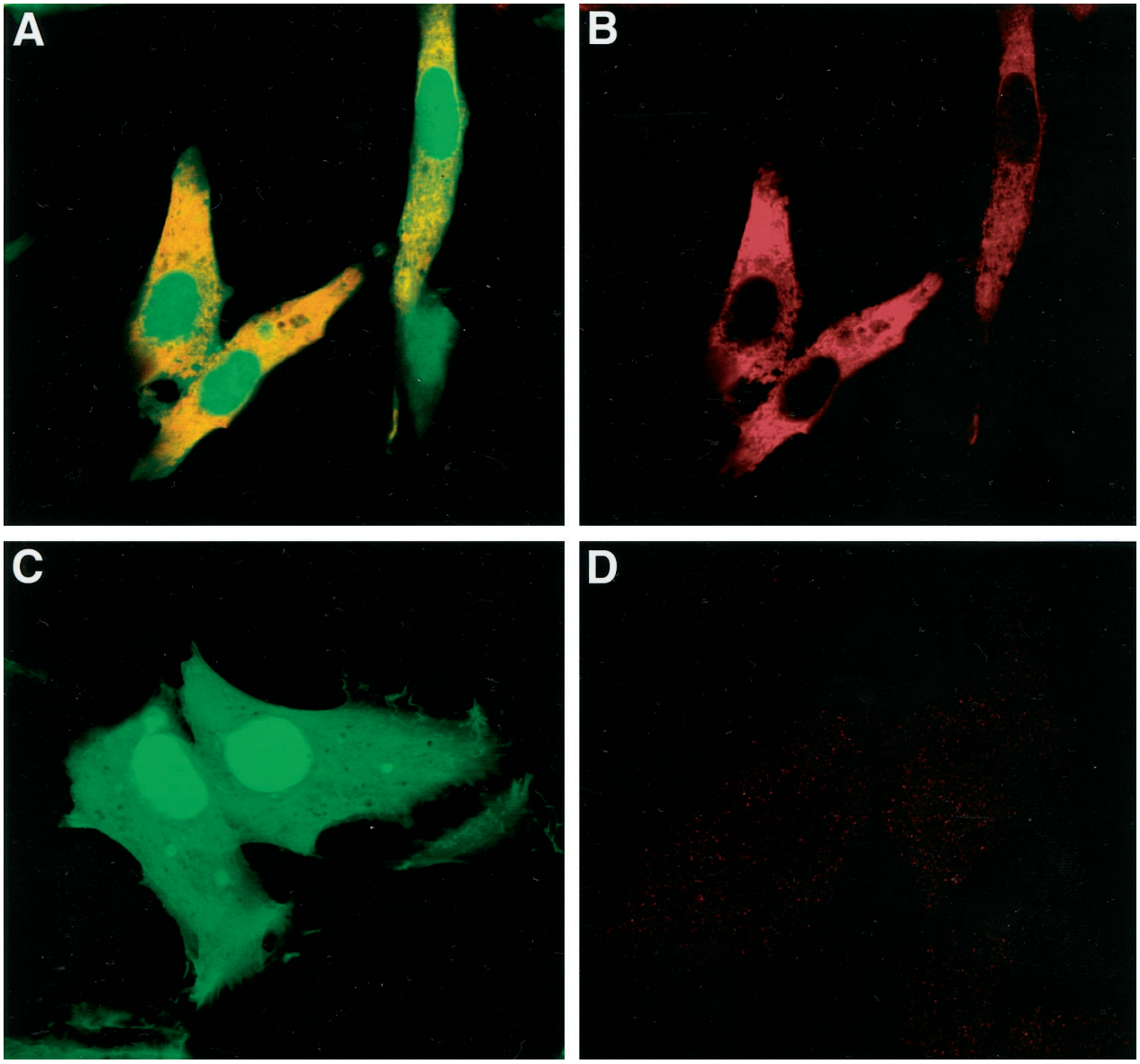

Figure 2. Detection by confocal microscopy of the intracellular accumulation of SDF-1β by the K15C mAb. TIGEF cells cotransfected with SDF-1β (or an insertless pcDNA3 plasmid) and GFP (green) expression vectors were incubated with K15C mAb and a secondary antibody conjugated to cye-3 (red). Cultures were treated with Brefeldine A. **A** and **B:** Cotransfection of SDF-1β and GFP expression vectors. **C** and **D:** Cotransfection of insertless pcDNA3 and a GFP expression vector. **A** and **C:** Simultaneous detection of GFP (green) and SDF-1β (red). **B** and **D:** Detection of SDF-1β (red).

SDF-1, facilitating their detection (Figure 1b). The ability of this antibody to specifically recognize SDF-1 products by indirect immunofluorescence in intact cells was also assessed. TIGEF cells were transfected with either SDF-1β cDNA or insertless DNA plasmids. Cotransfection of a GFP expression vector made it possible to identify cells that were efficiently transfected. Transfected cells were treated with Brefeldine A. Intracellular accumulation of SDF-1 was detected by immunofluorescence with K15C mAb in SDF-1β-transfected cells, whereas no signal was obtained in cells transfected with the insertless vector (Figure 2). Similar results were obtained when SDF-1 cDNAs were expressed in cell lines from simian or human origin (data not shown). The antibody failed to recognize any other chemokine currently identified by Western blot (data not shown).

SDF-1 Expression

In normal skin, SDF-1 immunostaining was uniformly present in blood vessels of different sizes. Small capillary vessels of the subpapillary plexus showed SDF-1 staining in ECs as well as in pericytes (Figure 3, C and D). Larger arteriolar and venular vessels in deeper skin layers displayed weak SDF-1 staining that was limited to ECs (Figure 3E). Preincubation of K15C mAb with SDF-1 peptide completely abolished immunostaining (data not shown). Expression of SDF-1 mRNA was confirmed in both ECs and pericytes by ISH with antisense SDF-1 riboprobes (Figure 4, C and D).

In both dermis and epidermis, scattered cells with cytoplasmic dendritic processes and typical DC morphology were strongly immunostained by anti SDF-1

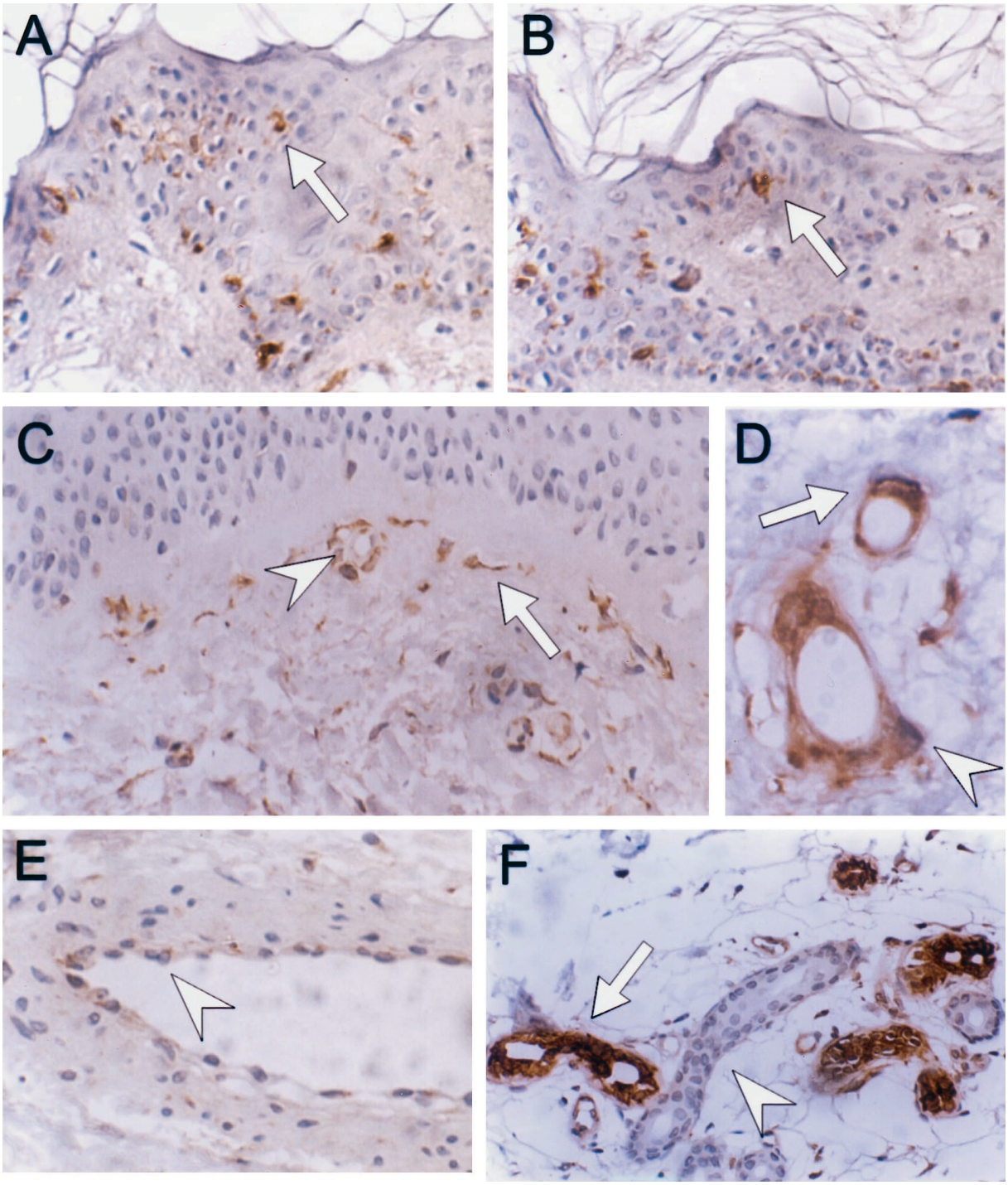

Figure 3. SDF-1 immunostaining in normal skin. **A:** Epidermal scattered DC were immunostained by anti-SDF-1 K15C MAb (**arrow**). **B:** A parallel section shows the identical morphology and distribution of SDF-1-positive and CD1a-positive epidermal DC (Langerhans cells). **C:** Dermis also contains scattered SDF-1 immunostained DC (**arrow**). EC and pericytes of dermal capillary vessels (**arrowhead**) were also immunostained by anti-SDF-1 MAb.**D:** Both EC (**arrow**) and pericytes (**arrowhead**) of small capillary vessels were immunostained. **E:** EC of larger vessels displayed weak immunostaining with anti-SDF-1 MAb. **F:** Epithelial cells of sweat glands showed strong SDF-1 immunostaining (**arrow**). Sweat gland immunostaining was not uniform, and there were unstained areas (**arrowhead**).

K15C MAb (Figure 3, A and C). These cells showed morphology and distribution identical to those of Langerhans cells immunostained with anti-CD1a antibody in serial consecutive sections (Figure 3 B). ISH with antisense SDF-1 riboprobes also demonstrated SDF-1 mRNA expression by scattered Langerhans cells (Figure 4 B). ISH with sense riboprobes did not produce any labeling above background (Figure 4 A).

Additional evidence corroborating the expression of SDF-1 in skin DCs was obtained from highly purified CD1a+ cell populations. CD1a+ DCs migrating out of skin explants were immunolabeled with the K15C mAb

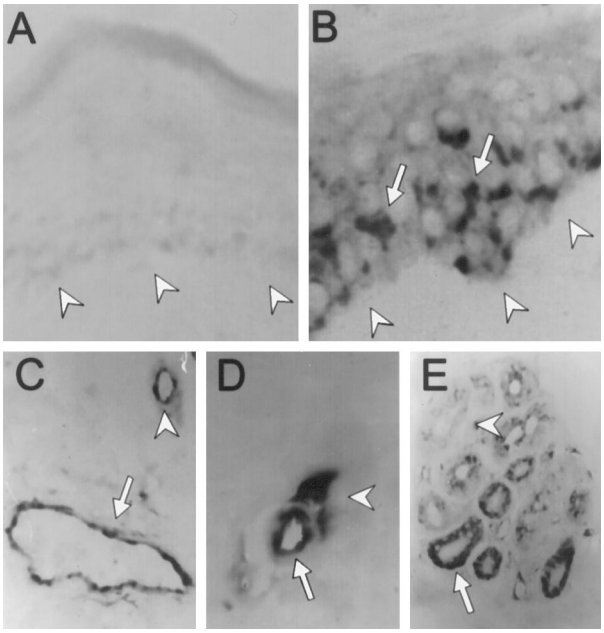

Figure 4. SDF-1 mRNA expression by ISH in normal skin. **A:** SDF-1 sense riboprobes did not produce any signal above background (**arrowheads** mark the dermoepidermal union). **B:** SDF-1 mRNA expression was detected in epidermal Langerhans cells (marked by **arrows**). **C:** Both small capillary vessels (**arrow**) and larger blood vessels (**arrowhead**) displayed EC labeling. **D:** A small capillary vessel with higher magnification shows labeling of both EC (**arrow**) and pericytes (**arrowhead**). **E:** Sweat gland epithelial cells showed uneven mRNA SDF-1 labeling, with alternating labeled (**arrow**) and unlabeled (**arrowhead**) areas.

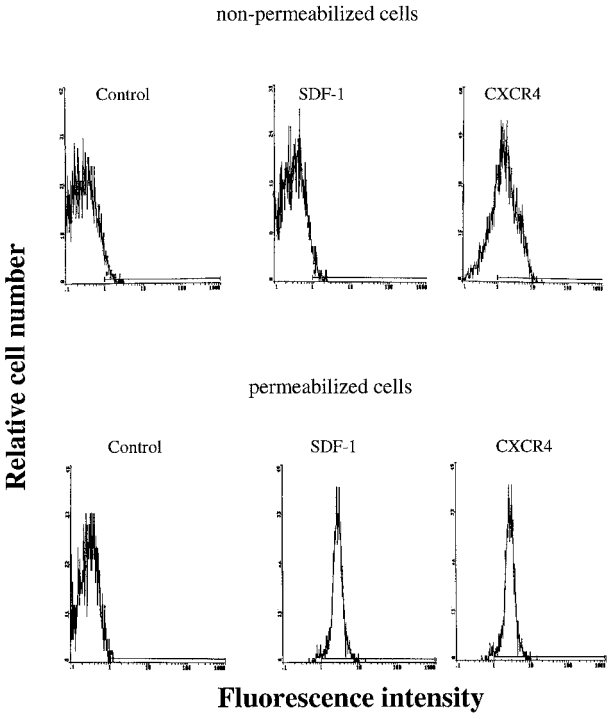

Figure 5. Expression of SDF-1 and CXCR4 in purified CD1a+ DC explanted from skin. Cytofluorometric analysis of CD1a+ cells labeled with the anti-SDF K15C mAb, 12G5 anti-CXCR4 mAb, or isotype-matched immunoglobulin (control). SDF-1 was only detected in permeabilized cells. CXCR4 was present at low levels on the surface of nonpermeabilized cells, and its detection increased in permeabilized cells.

and analyzed by FACS. Intracellular accumulation of SDF-1 was demonstrated in permeabilized cells, whereas no SDF-1 labeling was detected in nonpermeabilized cells (Figure 5). RT-PCR amplification confirmed the presence of SDF-1 mRNA in CD1a+ sorted DCs (Figure 6). Together, these findings demonstrate that skin DCs produce SDF-1 constitutively.

Epithelial cells of ecrine sweat glands and ductus were also strongly labeled by K15C mAb (Figure 3F). These structures were not uniformly stained, and different adjacent areas within the same gland showed discordant staining. SDF-1 immunostaining was not detected in keratinocytes. ISH showed the same mRNA pattern of expression as SDF-1 protein in sweat glands (Figure 4 E).

Normal skin fibroblasts were not labeled by anti-SDF-1 mAb or SDF-1 antisense riboprobes. In contrast, in sections from inflammatory skin diseases, fibroblasts close to inflammatory infiltrates showed strong SDF-1 immunostaining (Figure 7, A and C). The pattern was similar in scleroderma, cutaneous lupus, and dermatomyositis skin sections. In addition to fibroblasts, in areas close to or within inflammatory infiltrates, a high number of large mononuclear cells with monocyte-macrophage morphology displayed SDF-1 immunostaining (Figure 7, A and C). Small lymphocytes of inflammatory infiltrates were not stained by anti-SDF-1 mAb. This pattern was also confirmed by ISH with SDF-1 antisense riboprobes (data not shown). In cultured foreskin fibroblasts, but not in PBMCs, SDF-1 mRNA was also detectable by RT-PCR (Figure 6).

CXCR4 Expression

In normal skin, CXCR4 protein was detected in most cell types by immunohistochemistry with either 6H8 or 12G5 mAb. We found an identical staining pattern with the two antibodies. The strongest CXCR4 immunostaining was observed in ECs and pericytes of small capillary blood vessels and in epithelial cells of sweat glands (Figure 8, A and B). In larger vessels, EC but not smooth muscle cells were immunostained. Keratinocytes showed weaker positive CXCR4 immunostaining (Figure 8 A). Sweat glands were uniformly immunostained, contrasting with

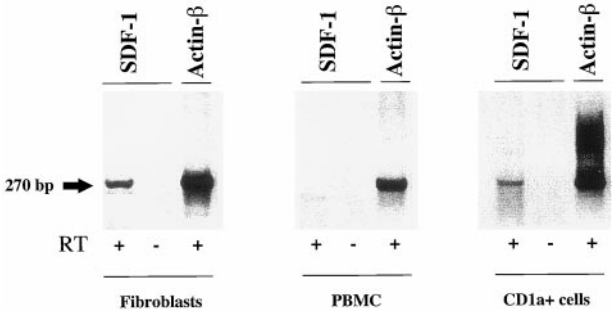

Figure 6. Detection of SDF-1 mRNA in CD1a+ DC explanted from the skin. Foreskin fibroblasts and PBMC were used, respectively, as sources of positive and negative controls for the expression of SDF-1 gene. cDNAs were generated by reverse transcription (RT) of total RNA and amplified by PCR. Aliquots of each sample were similarly processed for the expression of β-actin, which was used as a cDNA loading control. To rule out DNA contamination and verify the specificity of the PCR-amplified signal, addition of reverse transcriptase was omitted where indicated (RT).

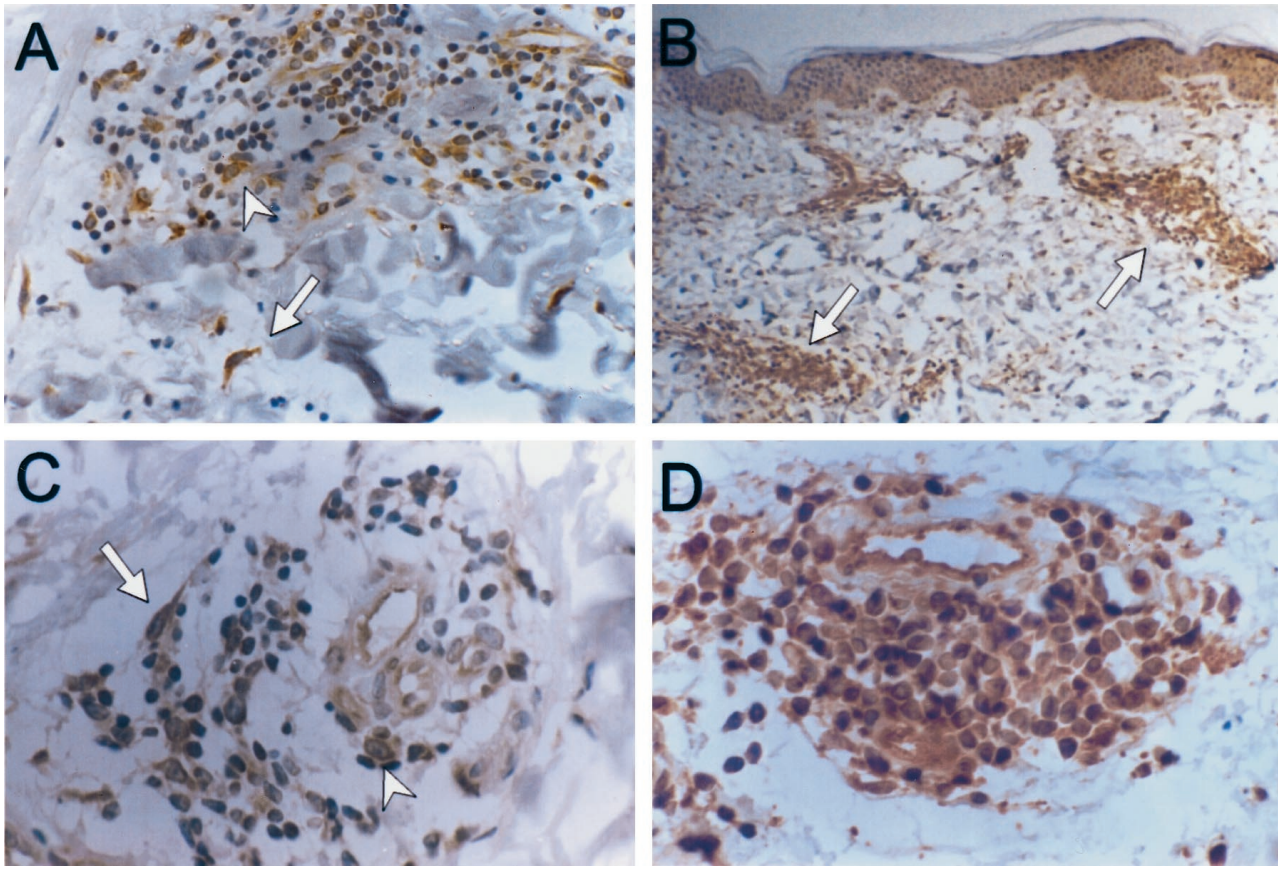

Figure 7. SDF-1 and CXCR4 immunostaining in inflammatory infiltrates from skin autoimmune diseases. **A**, **C:** SDF-1 immunostaining is present in a large number of fibroblasts (**arrows**) and large mononuclear cells (**arrowheads**) but not in small lymphocytes of inflammatory infiltrates. **B**, **D:** Virtually all lymphocytes and mononuclear cells within inflammatory infiltrates were CXCR4 positive. **A**, **C:** Cutaneous lupus. **B**, **D:** Dermatomyositis.

the pattern observed for SDF-1 (Figure 8 B). Some fibroblasts and dermal cells with dendritic morphology also displayed CXCR4 immunostaining (Figure 8, A and C). Because immunostaining does not differentiate between intracellular and membrane CXCR4, we analyzed surface CXCR4 expression in explanted DCs. Explanted DCs were immunolabeled with 12G5 mAb and analyzed by FACS. Low cell surface amounts of CXCR4 were detected in explanted CD1a+ DCs by FACS analysis of nonpermeabilized cells (Figure 5). Cell permeabilization permitted detection of a more intense CXCR4 staining of DCs, suggesting the preferential accumulation of the receptor in intracellular stocks.

In inflammatory infiltrates of all studied autoimmune skin diseases, virtually all mononuclear cells, including small lymphocytes, were strongly immunostained with anti-CXCR4 antibodies (Figure 7, B and D).

Discussion

The wide and constitutive expression of SDF-1 suggests a role in the homeostatic regulation of hemopoiesis and trafficking of mononuclear leukocytes rather than in inflammatory phenomena. This represents a difference from most other chemokines, the production of which is up-regulated by proinflammatory stimuli. Nevertheless, the potential function of SDF-1 in lymphoid cell recruitment to normal tissues other than bone marrow and liver or during inflammatory diseases has not been explored.

We here show that both SDF-1 and CXCR4 receptor are expressed by skin blood vessels of different sizes. Small capillary and larger venular and arteriolar vessels express SDF-1 protein. Given the capacity of ECs to internalize and accumulate chemokines from the extracellular environment,[21] we first hypothesized that expression of SDF-1 in these cells could merely result from uptake of the chemokine secreted by pericytes. This possibility was supported by the selective expression of SDF-1 mRNA observed in pericytes, but not in the adjacent ECs, from intestinal tract vessels of mice embryos.[8] However, we unambigously detect both the mRNA and product of the *SDF-1* gene in skin ECs. These divergent data could be due to species or tissue differences, or alternatively, they could be the consequence of a different pattern of SDF-1 expression during embryogenesis. The abnormal development of gastrointestinal vascularization observed in CXCR4 knock-out mice[8] and the capacity of cultured ECs to express CXCR4 and respond to SDF-1[25,26] suggest a role for the autocrine secretion of SDF-1 in the homeostasis of vascular endothelium.

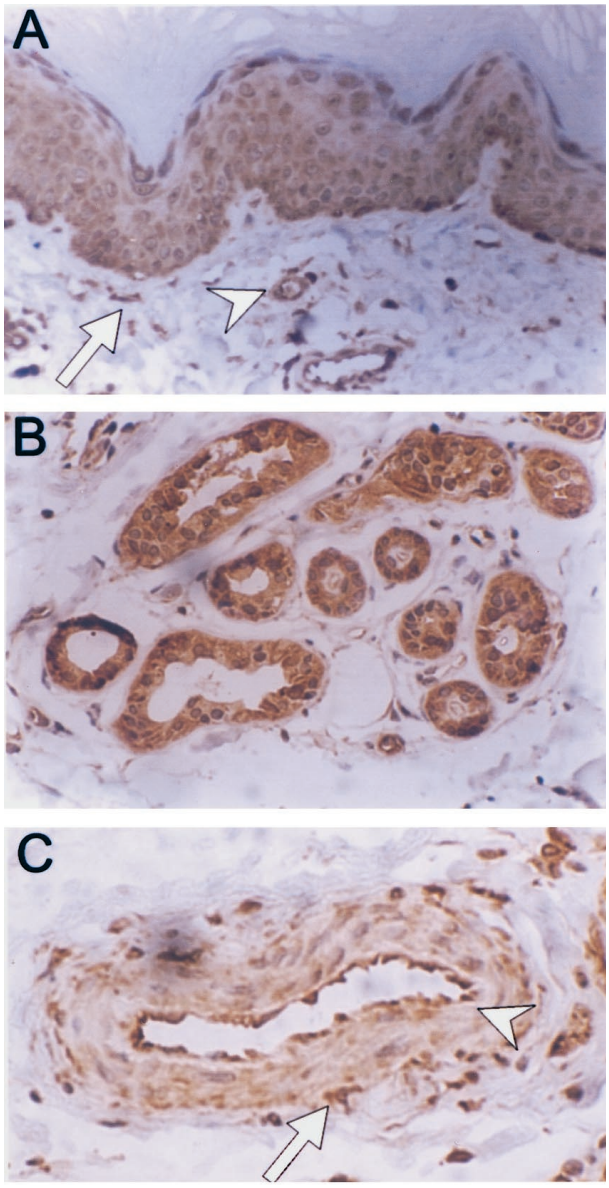

Figure 8. CXCR4 immunostaining in normal skin. **A:** Normal epidermal keratinocytes were uniformly stained by anti-CXCR4 antibodies. EC and pericytes (**arrowhead**) and dermal fibroblasts (**arrow**) were also immunostained. **B:** Normal sweat gland epithelial cells were strongly and uniformly immunostained. **C:** Larger vessels also showed strong CXCR4 staining (**arrowhead**). Cells with dendritic (**arrow**) and fibroblastic morphology around blood vessels are also immunostained.

Whether this function is mediated by SDF signaling on ECs or by other cell populations activated by the chemokine is not known.

The production of SDF-1 by ECs or DCs raises questions on the autocrine responsiveness of these cells to SDF-1 *in vivo*. It is possible that *in vivo*, the autocrine constitutive secretion of the chemokine leads to down-regulation and desensitization of CXCR4 in both cell types. Thus a function of SDF-1 secreted by these cell types might be to induce haptotactical attraction of CXCR4+ leukocytes and participate in the regulation of their trafficking from blood to tissues. In keeping with this assumption, it should be noted that SDF-1 has the highest capacity described among chemokines to promote the attraction and arrest of circulating T lymphocytes on the luminal side of vascular endothelium.[27] Moreover, tissue extravasation of circulating blood CD4 lymphocytes in transgenic mice with enforced CXCR4 expression in this T-cell subpopulation has been reported.[28] Furthermore, when injected into nontransgenic animals, transgenic CD4 lymphocytes overexpressing CXCR4 rapidly leave the blood. This phenomenon is pertussis-toxin-sensitive, which indicates that, like SDF-1-mediated activation, it depends on heterotrimeric G protein-mediated cell signaling.[28] It is conceivable that the disappearance of transgenic CXCR4+ T lymphocytes from blood could be due to interaction of the overexpressed receptor with SDF-1 produced by ECs.

Continuous exposure of circulating human blood T lymphocytes to SDF-1 may be related to the reduced cell surface expression of CXCR4 observed in these cells.[29,30] Interestingly, with isolation and short incubation *in vitro,* human blood T lymphocytes, which do not express the *SDF-1* gene, redistribute CXCR4 to the cell membrane from preexisting intracellular pools of the protein.[29] Binding to CXCR4 of either soluble[15,16] or EC membrane-bound SDF-1 could be responsible for the reversible cell surface down-regulation of CXCR4 in peripheral T lymphocytes.

We have recently shown that both SDF-1α and β bind in a CXCR4-independent manner to cell membrane heparan sulfates (HS), including those of EC lines. Interaction of SDF-1 with HS is mediated by a cluster of basic amino acids (Lys^{24} His^{25} Lys^{26}) located in the first β-strand of the chemokine.[31] Interestingly, while associated with EC heparan sulfates, the amino-terminal domain of SDF-1, which is recognized by the mAb K15C and is required for binding to and activation of CXCR4, remains exposed and free for interaction with the receptor. Together, these findings suggest that *in vivo*, the functional form of the chemokine secreted by ECs might be a proteoglycan-immobilized SDF-1 expressed on the EC luminal side of sinusoid endothelia.

Although the constitutive expression of SDF-1 by endothelium and skin DCs does not support a primary proinflammatory chemoattractant function for this factor, the specific pattern of SDF-1 expression observed in the skin from autoimmune inflammatory diseases makes it possible to envisage its participation in these pathological phenomena. Whereas no SDF-1 mRNA or protein was observed in normal dermal fibroblasts, fibroblasts close to or within dermal inflammatory infiltrates strongly express SDF-1. Fibroblasts have been found to express many other chemokines in response to a variety of proinflammatory stimuli and may therefore represent a source of chemoattractants in connective tissue before infiltration by nonresident cells occurs.[32] A recent report shows that the *SDF-1* gene is induced in serum-activated fibroblasts.[33] In keeping with these findings, we detect SDF-1 mRNA in human fibroblasts cultured with serum. It can be speculated that the expression of SDF-1 observed in dermal fibroblasts from autoimmune inflammatory disorders is related to the pathological activation status of these cells. In addition to fibroblasts, infiltrating mononu-

clear cells of monocyte-macrophage morphology also express SDF-1. Because peripheral blood leukocytes do not express SDF-1 constitutively,[3] the expression of SDF-1 by mononuclear cells in inflammatory infiltrates is probably induced and deserves further analysis. Overall, these findings open the possibility that SDF-1 produced by fibroblasts and the accompanying mononuclear cells takes part in the pathogenesis of autoimmune inflammatory skin diseases.

In normal skin, DCs express SDF-1 mRNA and protein. DCs are a well-defined system of immune surveillance in many tissues. Expression of SDF-1 by DCs may be a mechanism for attracting naive T lymphocytes, which typically express CXCR4 receptor,[34,35] into close contact with these antigen-presenting cells.[36] Tissular extravasation of circulating naive T lymphocytes to be attracted by resident DCs could be ensured under physiological circumstances by SDF-1 constitutively produced by ECs.

Besides these physiological functions, the expression of CXCR4 in DCs and their autocrine secretion of SDF-1 could provide some clues to the preferential transmission of R5 and delayed propagation of X4 HIV isolates in infected individuals.[17] Cells from DC lineage are thought to support the primary infection and replication of SIV in exposed mucosas,[37] which appears to be a preferential site for early virus replication[38] and CD4 T lymphocyte depletion,[39,40] even when the viruses are inoculated intravenously.[39] By using Langerhans cells from skin, which are immature DCs resembling resident DCs of intestinal and genital mucosa, it has been shown that this type of cell is not susceptible to infection by X4 HIV isolates, although it permits replication of R5 HIV viruses.[19,41] Infection of DCs by X4 viruses strikingly and directly correlates with their CXCR4 cell surface expression. Interestingly, it has been shown that CXCR4 expression in resident skin DCs is preferentially intracellular.[19] Given the production of SDF-1 by DCs, it is conceivable that the occupancy and the consecutive cell surface down-regulation of CXCR4 induced by the autocrine secretion of the chemokine may account for the reduced cell surface expression and intracellular accumulation of CXCR4. Thus SDF-1-down-regulated expression of CXCR4 in DCs may explain the refractory environment that they offer to X4 HIV strains. In addition, secretion of SDF-1 could also selectively interfere with transmission of X4 HIV viruses to DC-neighboring CD4 T lymphocytes, thus limiting their propagation. Our unpublished observations demonstrate that SDF-1 expression extends to DCs of lymph nodes, suggesting that this can be a general feature of DCs. It will be very important to determine whether SDF-1 production by DCs in lymphoid tissues has the capacity to interfere with X4 propagation within these organs.

In conclusion, this study provides new data on the identification of cells expressing the *SDF-1* gene and producing SDF-1 protein. Our findings shed light on the physiological functions proposed for the SDF-1/CXCR4 system and make it possible to envisage its participation in pathological processes associated with inflammatory disorders or HIV infection. Further characterization of SDF-1 tissue expression and identification of cell types producing the chemokine will be critical to understanding the role played by the SDF-1/CXCR4 system in both physiological and pathological situations during postnatal life.

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References

1. Baggiolini M, Dewald B, Moser B: Human chemokines: an update. Annu Rev Immunol 1997, 15:675–705
2. Nagasawa T, Tachibana K, Kawabata K: A CXC chemokine SDF-1/PBSF. A ligand for a HIV receptor, CXCR4. Adv Immunol 1999, 71:211–228
3. Shirozu M, Nakano T, Inazawa J, Tashiro K, Tada H, Shinohara T, Honjo T: Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF-1) gene. Genomics 1995, 28:495–500
4. Nagasawa T, Kikutani H, Kishimoto T: Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc Natl Acad Sci USA 1994, 91:2305–2309
5. Nagasawa T, Hirota S, Tachibana K, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T: Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996, 382:635–638
6. Zou Y, Kottmann AH, Kuroda M, Taniuchi I, Littman DR: Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998, 393:595–598
7. Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, Broson RT, Springer TA: Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci USA 1998, 95:9448–9453
8. Tachibana K, Hirota S, Iizasa H, Yoshida N, Kawabata K, Kataoka Y, Kitamura Y, Matsushima K, Yoshida N, Nishikawa S, Kishimoto T, Nagasawa T: The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature 1998, 393:591–594
9. Bleul C, Fuhlbrigge R, Casanovas JM, Aiuti A, Springer T: A highly efficacious lymphocyte chemoattractant stromal cell-derived factor 1 (SDF-1). J Exp Med 1996, 184:1101–1109
10. Hamada T, Mohle R, Hesselgesser J, Hoxie J, Nachman RL, Moore MA, Rafii S: Transendothelial migration of megakaryocytes in response to stromal cell-derived factor 1 (SDF-1) enhances platelet formation. J Exp Med 1998, 188:539–548
11. Mohle R, Bautz F, Rafii S, Moore MA, Brugger W, Kanz L: The chemokine receptor CXCR-4 is expressed on CD34+ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. Blood 1998, 91:4523–4530
12. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T: Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 1999, 283:845–848
13. Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, Schwartz O, Heard JM, Clark-Lewis I, Legler DF, Loetscher M, Baggiolini M, Moser B: The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 1996, 382:833–835
14. Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA: The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature 1996, 382:829–832
15. Amara A, Le Gall S, Schwartz O, Salamero J, Montes M, Loetscher P, Baggiolini M, Virelizier JL, Arenzana-Seisdedos F: HIV coreceptor downregulation as antiviral principle: SDF-1α-dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. J Exp Med 1997, 186:139–146
16. Signoret N, Oldridge J, Pelchen-Matthews A, Klasse PJ, Tran T, Brass

LF, Rosenkilde MM, Schwartz TW, Holmes W, Dallas W, Luther MA, Wells TN, Hoxie JA, Marsh M: Phorbol esters and SDF-1 induce rapid endocytosis and down modulation of the chemokine receptor CXCR4. J Cell Biol 1997, 139:651–664
17. Moore JP, Trkola A, Dragic T: Co-receptors for HIV-1 entry. Curr Opin Immunol 1997, 9:551–562
18. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, Dean M, Honjo T, Tashiro K, Yabe D, Buchbinder S, Vittinghoff E, Goedert JJ, O'Brien TR, Jacobson LP, Detels R, Donfield S, Willoughby A, Gomperts E, Vlahov D, Phair J, O'Brien SJ: Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort. Science 1998, 279:389–393
19. Zaitseva M, Blauvelt A, Lee S, Laqpham CK, Klaus-Kovtum V, Mostowski H, Manischevitcz J, Golding A: Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. Nat Med 1997, 3:1369–1375
20. Zoeteweij JP, Golding H, Mostowski H, Blauvelt A: Cytokines regulate expression and function of the HIV coreceptor CXCR4 on human mature dendritic cells. J Immunol 1998, 161:3219–3223
21. Middleton J, Neil S, Wintle J, Clark-Lewis I, Moore H, Lam C, Auer M, Hub E, Rot A: Transcytosis and surface presentation of IL-8 by venular endothelial cells. Cell 1997, 91:385–395
22. Mondor I, Moulard M, Ugolini S, Klasse PJ, Hoxie J, Amara A, Delaunay T, Wyatt R, Sodroski J, Sattentau QJ: Interactions among HIV gp120, CD4, and CXCR4: dependence on CD4 expression level, gp120 viral origin, conservation of the gp120 COOH- and NH_2-termini and V1/V2 and V3 loops, and sensitivity to neutralizing antibodies. Virology 1998, 248:394–405
23. Da SilvaTexeira MF, Lambert V, Mselli-Lakahl L, Chettab A, Chebloune Y, Mornex JF: Immortalization of caprine fibroblasts permissive for replication of small ruminant lentiviruses. Am J Vet Res 1997, 58:579–584
24. Larsen CP, Steinman RM, Witmer-Pack M, Hankins OF, Morris PJ, Austyn JM: Migration and maturation of Langerhans cells in skin transplants and explants. J Exp Med 1990, 172:1483–1493
25. Volin MV, Joseph L, Shockley MS, Davies PF: Chemokine receptor CXCR4 expression in endothelium. Biochem Biophys Res Commun 1998, 242:46–53
26. Feil C, Augustin HG: Endothelial cells differentially express functional CXC-chemokine receptor-4 (CXCR-4/fusin) under the control of autocrine activity and exogenous cytokines. Biochem Biophys Res Commun 1998, 247:38–45
27. Campbell JJ, Hedrick J, Zlotnik A, Siani MA, Thompson DA, Butcher EC: Chemokines and the arrest of lymphocytes rolling under flow conditions. Science 1998, 279:381–384
28. Sawada S, Gowrisankar K, Kitamura R, Suzuki M, Suzuki G, Tahara S, Koito A: Disturbed CD4 (+) cell homeostasis and in vitro HIV susceptibility in mice expressing T cell line tropic HIV-1 receptors. J Exp Med 1998, 187:1439–1449
29. Bermejo M, Martin-Serrano J, Oberlin E, Pedraza MA, Serrano A, Santiago B, Caruz A, Loetscher P, Baggiolini M, Arenzana-Seisdedos F, Alcami J: Activation of blood T lymphocytes down-regulates CXCR4 expression and interferes with propagation of X4 HIV strains. Eur J Immunol 1998, 28:3192–3204
30. Jourdan P, Abbal C, Nora N, Hori T, Uchiyama T, Vendrell JP, Bousquet J, Taylor N, Pene J, Yssel H: IL-4 induces functional cell-surface expression of CXCR4 on human T cells. J Immunol 1998, 160:4153–4157
31. Amara A, Lorthioir O, Valenzuela A, Magerus A, Thelen M, Montes M, Virelizier JL, DelePierre M, Baleux F, Lortat-Jacob H, Arenzana-Seisdedos F: Stromal cell derived factor-1α associates with heparan sulfates through the first β-strand of the chemokine. J Biol Chem 1999, 274:23916–23925
32. Smith RS, Smith TJ, Blieden TM, Phipps RP: Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. Am J Pathol 1997, 151:317–322
33. Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, Lee JCF, Trent JM, Staudt LM, Hudson J Jr, Boguski MS, Lashkari D, Shalon D, Botstein D, Brown PO: The transcriptional program in the response of human fibroblasts to serum. Science 1999, 283:83–87
34. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A: Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 1998, 187:875–883
35. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrossio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, Sinigaglia F: Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998, 187:129–134
36. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 1998, 392:245–252
37. Pope M, Elmore D, Ho D, Marx P: Dendrite cell-T cell mixtures, isolated from the skin and mucosae of macaques, support the replication of SIV. AIDS Res Hum Retroviruses 1997, 13:819–827
38. Spira AL, Marx PA, Patterson BK, Mahoney J, Koup RA, Wolinsky SM, Ho DD: Cellular targets of infection and route of viral dissemination following an intravaginal inoculation of SIV into rhesus macaques. J Exp Med 1996, 183:215–225
39. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC, Lackner AA: Gastrointestinal tract as a major site of $CD4^+$ T cell depletion and viral replication in SIV infection. Science 1998, 280:427–431
40. Clayton F, Snow G, Reka S, Kotler DP: Selective depletion of rectal lamina propria rather than lymphoid aggregate CD4 lymphocytes in HIV infection. Clin Exp Immunol 1997, 107:288–292
41. Reece JC, Handley AJ, Anstee EJ, Morrison WA, Crowe SM, Cameron PU: HIV-1 selection by epidermal dendritic cells during transmission across human skin. J Exp Med 1998, 187:1623–1631